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THE BIOSYNTHESIS OF VITAMINS

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THE BIOSYNTHESIS OF VITAMINS

The fact that vitamins may be synthesized within the animal body is a startling concept. At first examination it appears to be at complete variance with the original definition of the vitamins—*essential substances which must be provided in the diet because the body is unable to manufacture them.* As the vitamins have emerged from their early status as obscure and somewhat mysterious materials to become identified as specific chemical compounds, many of which the chemist can synthesize at will in the laboratory, it has become possible to study their chemical behavior in the body and to relate their activities to specific biochemical processes. With the growing understanding of the mechanisms of vitamin action and of variations in species requirements, the concept of vitamin biosynthesis has not only been found to be compatible with earlier theories, but indeed has been the means of shedding new light on the behavior of the vitamins. That vitamins must be provided in the diet remains axiomatic; that in addition, they may be synthesized in the body by microorganisms normally inhabiting the body is also a fact. *In vivo* bacterial activity resulting in vitamin synthesis does not alter the fact that vitamins in food remain a vital dietary need. However, it may necessitate a reevaluation of dietary vitamin requirement standards and, depending on the degree and nature of such activity and on the diet, may either precipitate or prevent symptoms of vitamin deficiencies.

Vitamin biosynthesis may occur as a function of the tissues or as a product of microbial activity, inside or outside the body. The present discussion will be limited to the biosynthesis of vitamins by microorganisms inhabiting the intestinal tract of various animals. The discovery of the sulfomamides as effective bacteriostatic agents has played a major role in establishing and clarifying the significant new concept of *in vivo* synthesis of vitamins by intestinal bacteria.

Bacterial Synthesis of Vitamins:

Long before the vitamin concept was born, Louis Pasteur (1) pioneer microbiologist, expressed the belief that bacteria present in the intestinal tract exercise an important, perhaps vital function in the animal economy. About 30 years later, Pacini and Russell (2) demonstrated that typhoid bacteria contributed to the cultures in which they were grown a factor which stimulated the growth of rats. Shortly before that, Bottomley (3), a British plant physiologist, had observed that the addition of bacteria to fertilizer stimulated the growth of seedlings. In the 30-year interval between Pasteur's statement and the initial demonstrations of the ability of bacteria to manufacture growth stimulants, numerous experiments were conducted which fostered the suspicion that intestinal microbes contributed significantly to

the nutrition of the host animal. Experiments with guinea pigs, toads and chicks reared in sterile environments and fed sterile food ended with impairment of growth and ultimate death of the animals within a few weeks (4, 5, 6, 7, 8). The inoculation of ailing and poorly growing chicks with cultures of colon bacilli resulted in prompt recovery (8).

In 1911, Osborne and Mendel (9) reported that rats on purified diets grew poorly and resorted to coprophagy—ingestion of feces—with the surprising result that weight gains were rapidly restored to normal. It was concluded that the ingested feces (from normal animals on a balanced stock diet) contained a growth-stimulating factor. Nearly ten years later, two French scientists, Portier and Randoin (10) demonstrated that the mysterious growth factor was present not only in feces of healthy animals, but in those of deficient animals as well. Their work thus eliminated the possibility that the growth factor was excreted, either as an unutilized or excess dietary component, and pointed strongly to the synthesis of a new material somewhere in the intestinal tract. A further link in the chain of growing evidence was added a few years later when Steenbock, Sell and Nelson (11) observed that deficient rats recovered when allowed access to their own feces or when provided with a supplement of "vitamin B"* in yeast. This seemed to indicate that the important factor in feces was either closely related to, or identical with "vitamin B". This coincided with the earlier thesis of Cooper (12) who suggested, soon after Osborne and Mendel's work, that bacterial action in the intestinal tract probably had resulted in the synthesis of the vitamin. The theory subsequently was tested and corroborated by many other workers.

The Phenomenon of Refection:

During the 15-year period 1911-1926, progressive researches established that experimental animals were benefited by the ingestion of feces. Later, these benefits were attributed correctly to the presence of vitamins in the feces. The origin of the vitamins subsequently was traced to the activity of microorganisms in the intestinal tract. In 1926 these concepts were extended greatly with the introduction of the theory of *refection.*

The phenomenon of refection was first observed by Fridericia (13) who noted that in a group of animals on a deficient diet some spontaneously recovered without the addition of vitamin B and without resort to coprophagy. This mechanism described as "refection", was considered to be the result of a change in the intestinal flora which enabled a "refected" animal to thrive and grow without vitamin B in the diet. The change in

*The vitamin B complex and its numerous components were not yet known; "vitamin B" referred to a presumably single substance.

intestinal flora occurred spontaneously in some animals but could be transmitted readily to other animals by implantation of the vitamin-producing microorganisms resulting through the feeding of feces. The phenomenon of spontaneous refection remained largely unclarified, but it led to the enunciation of a concept which was destined to exert a profound influence on the significance of intestinal bacteria in the nutrition of higher animals. The concept was that vitamins not only might be synthesized by bacteria in the gastro-intestinal tract, but actually might be released into the surrounding medium in sufficient quantities to be absorbed and utilized by the host animal.

Influence on Dietary Requirements: The concept of intestinal synthesis of vitamins by microorganisms cleared up many long-standing questions as to why and how certain animals were able to grow and thrive on diets severely deficient in the vitamin B complex. This was particularly true in the case of ruminants which unlike any other species studied, possessed the strange ability of being able to grow to maturity, give birth to normal offspring and produce normal supplies of milk on a ration almost devoid of the B complex. Soon after Fridericia's work, Bechdel *et al* (14, 15, 16), investigating this unique behavior of cattle, demonstrated two interesting facts: First, that contents of the rumens or first stomachs of cattle on vitamin B-deficient diets actually contained vitamin B; and second, that one of the more prevalent microorganisms (genus, *Flavobacterium*) found in the bacterial flora of the rumen was capable of synthesizing vitamin B in test tubes outside the body. With the expanding knowledge concerning the multiple nature of "vitamin B", more detailed studies of the synthesis of individual members of the B complex were undertaken by various groups. It now has been demonstrated that virtually all of these—thiamin, riboflavin, biotin, pyridoxine, pantothenic acid, nicotinic acid, folic acid—are synthesized in the rumen of sheep or cows in amounts sufficiently large to permit these animals to dispense with dietary sources of these vitamins. This, however, does not apply to very young animals of these species in which the rumen is not developed.

That intestinal synthesis of vitamins occurs to some extent in non-ruminants as well was reported by Guerrant, Dutcher and their associates (17, 18, 19, 20), who demonstrated the synthesis of B vitamins in the cecum of rats fed diets containing dextrinized starch or the milk sugar, lactose. In commenting on the difference between rats and the ruminant species they said (19):

> "The cow is not unique in its ability to synthesize the B vitamins in its digestive tract. The cow does have the advantage over the rat in that the vitamins are synthesized higher up in the alimentary canal of the cow (rumen), thus enabling

this animal to secure (on a ration deficient in the B vitamins) full benefit of the synthesized products without the necessity of reingestion of feces."

In man, as in other non-ruminants, bacterial synthesis of vitamins in the intestinal tract plays a significant, although generally minor role in determining nutritional requirements from outside sources. Under certain circumstances, however, interference with such synthesis over a prolonged period may be sufficient to alter dietary requirements materially and indeed, to precipitate obvious symptoms of vitamin deficiencies.

Factors Influencing Intestinal Synthesis:

The synthesis of vitamins by intestinal microorganisms may be influenced in many ways. A marked change in the internal environment may inhibit the activity of the intestinal microflora to such an extent that vitamin synthesis may be blocked completely; on the other hand the introduction of substances which are favorable to the growth of these microorganisms tends to increase their number and, consequently, the total synthesis of vitamins. Food factors—proteins, fats, carbohydrates, vitamins and minerals—are among the most significant influences. More recently the relatively insoluble sulfonamides, because of their bacteriostatic effects, have been found to play an extremely significant role in the phenomenon of intestinal vitamin synthesis.

Significance of Intestinal Synthesis:

That bacterial synthesis of vitamins does occur in the intestinal tract of animals to an appreciable extent has been established unequivocally. The actual benefit to the animal from such synthesis has not yet been determined with any degree of precision. Factors influencing this include the specific quantity of vitamin which may be synthesized within the intestinal tract of a particular animal species; the location in the intestinal tract of such synthesis; and finally, the differences in absorption by the tissues of the various vitamins synthesized. All of these factors must be determined and carefully evaluated before it may become possible to determine the significance or full value of intestinal vitamin synthesis for a given animal.

Quantity: The amount of a particular vitamin synthesized is of considerable importance in determining the vitamin economy of the animal. Frequently the amount synthesized is sufficiently large to take care of all of the animal's requirements for that particular vitamin. In other instances, however, the vitamin synthesized may be so small in quantity as to exert a negligible effect on the requirement from outside dietary sources. Thus, for example, ruminants may have their entire B complex requirements provided by intestinal synthesis and therefore are not dependent upon external dietary sources of these vitamins. In other species the extent of vitamin

synthesis may be negligible so that the animal is entirely dependent upon dietary sources. In still other instances, the amount of vitamin synthesized may be so close to that required by the organism that in a group of animals under identical conditions on a marginal diet, some would develop a deficiency while others would not. It has been shown, for example, that in a group of chicks on a vitamin K-deficient diet some will develop hemorrhages and others will not, while still others may recover spontaneously after having exhibited symptoms of vitamin K deficiency (21). Similar observations have been reported with respect to mice maintained on inositol-free diets (22, 23).

Location of Synthesis: Regardless of quantity synthesized, the animal will obtain little or no benefit unless the synthesized vitamin is actually absorbed into the system and utilized. A prime factor in determining the availability of intestinally synthesized vitamins is the location of such synthesis in the intestinal tract. As previously pointed out, if the vitamins are synthesized high up in the intestinal tract where absorption is maximal, the animal will derive full benefit from the vitamins; if the vitamins are synthesized in the lower portion of the intestinal tract where absorption proceeds at a minimum, bacterial synthesis of vitamins will have very little significance with respect to fulfilling essential requirements. This, in addition to the quantity synthesized, accounts for the effectiveness of B complex synthesis in ruminants. It explains, further, why vitamin synthesis in non-ruminants and in immature ruminants in whom the rumen has not yet developed fully cannot adequately meet the vitamin requirements of the animal, so that such species are almost completely dependent upon diet for their vitamin supplies.

Variations in Absorption: Still another factor to be considered in evaluating the results of intestinal vitamin synthesis is the variability in the absorption of the different vitamins. Najjar and Holt (24) cite the examples of biotin, folic acid, pantothenic acid and pyridoxin all of which have been shown to be synthesized in the cecum of the rat (25). Deficiencies of biotin and of folic acid can be produced only with the aid of the sulfonamides. Simple dietary deprivation will precipitate deficiencies of pantothenic acid and pyridoxin. This has been interpreted to signify that intestinally synthesized biotin and folic acid are absorbed readily and in adequate amounts, so that the animal is independent of dietary supplies. Pantothenic acid and pyridoxin, however, although similarly synthesized in substantial quantities, are not as well absorbed since a dietary deprivation of either of these will result in a deficiency. This explanation holds true, it is pointed out, provided the requirements of pantothenic acid and pyridoxin are not well beyond such amounts as are normally synthesized.

The Role of the Sulfonamides:

Sulfanilamide, grandparent of the group of modern drugs included under the term, sulfonamides, is an organic chemical which has been known for many years. It was not until relatively recently, however, that the antibiotic properties of this compound and its derivatives became known. The sulfonamides interfere with the metabolism of microorganisms, preventing their growth and multiplication. When these antibiotic properties are exerted upon bacteria in the intestinal tract, an important consequence is the impairment of vitamin synthesis resulting from the normal activities of certain of the bacterial inhabitants.

An important mechanism which appears to be involved in this metabolic interference is the competitive action between many of the sulfonamides and the compound *p*-aminobenzoic acid*. The story of the competitive antagonism between sulfanilamide (*p*-aminobenzoic sulfonamide) and the structurally related compound, *p*-aminobenzoic acid constitutes one of the most fascinating chapters in modern microbiology, and has led to the development of radically new concepts in bacteriostasis and chemotherapy. Much of this material is beyond the scope of this Review which is concerned merely with the basic principle involved: The interference with essential metabolic reactions by means of inhibitory compounds (sulfonamides, in this case) modelled on essential metabolites (in this case, *p*-aminobenzoic acid). The mode of interference has been likened to the behavior of two keys with similar, but not identical notches. Both keys will fit into the lock, but only the correctly notched key will turn the lock. The other key, because it so closely resembles the correct key and fits into the lock, blocks the lock by its presence and prevents the correct key from working. In this way the sulfonamides substitute for an essential metabolite and thereby prevent its utilization by the microorganism.

The sulfonamides may be classified into two groups, those which are soluble (sulfanilamide, sulfathiazole, sulfadiazine) and those which are relatively insoluble (sulfaguanidine, sulfasuxidine). It is the latter group which is of importance as intestinal bacteriostatic agents because, being insoluble, they are not absorbed, but remain in the intestinal tract and exert their maximal antibiotic activity.

The sulfonamides have a dual significance in nutrition problems. The prolonged therapeutic use of the insoluble sulfonamides may precipitate marked vitamin deficiencies in the patient since essential vitamin-producing microorganisms are eliminated along with the disease-producing bacteria.

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*The vitamin nature of *p*-aminobenzoic acid, another well-known organic compound, was discovered indirectly when it was found that this compound reversed the bacteriostatic activity of sulfanilamide.

This makes necessary a careful evaluation of the patient's vitamin intake and may indicate supplementation of the diet with B vitamins and vitamin K to prevent the development of lesions associated with deficiencies of these vitamins. Second, and of primary interest with respect to nutrition research, is the fact that sulfonamides may be used as a tool to induce vitamin deficiencies at will in experimental animals and thereby aid in the investigation of these nutritional disturbances. The sulfonamides also provide a means of eliminating the complicating factor of intestinal synthesis during the course of experiments dealing with the vitamin-deficient diets. Some of the nutrition studies made possible with the aid of the sulfonamides will be described in the forthcoming issue of this Review.

Conclusion:

The phenomena of vitamin synthesis by bacteria, both *in vitro* and within the animal body, have been demonstrated conclusively. As a result, a new perspective has been given to considerations of animal and human vitamin requirements and to the understanding of vitamin deficiency diseases. Advances in microbiology dealing with the nutritional requirements and metabolism of bacteria and with principles of bacteriostatic chemotherapy through competitive interference with metabolic processes have played an outstanding role in the development of the concept of vitamin biosynthesis and in the recognition of its significance in the economy of the animal organism. The implications of intestinal vitamin synthesis in animals already have been recognized and are well defined, although knowledge in this field is still meager. Some beginnings already have been made in relating these findings to the nutritional requirements of human beings and preliminary studies on the phenomena of vitamin biosynthesis in man already have been reported (24).

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NEWS DIGEST

TEMPERATURE EFFECTS ON STORED VEGETABLES—The practice of holding fresh fruits and vegetables in cold storage to prevent spoilage is based on the belief that low temperatures retard the biological processes which bring about aging and decay. Although this theory is for the most part true, recent studies have demonstrated that certain biological processes not only are able to continue at low temperatures but may even be accelerated under such conditions. Thus citrus fruits kept at 32° to 40° F. (temperatures above the freezing points of these fruits) for several weeks develop pitting and discoloration of the rind; "watery breakdown," a disorder giving the fruit the appearance of having been frozen; as well as other blemishes of the rind or internal membranes. Temperature ranges for the various disorders have been found to be quite highly specific. For example, discoloration of lemon membrane is more severe at 36° and 40° than at 32°, 50° or 60°. Tropical fruits such as pineapples, papayas, bananas and even tomatoes, when held at low temperatures, will suffer an interruption of their metabolic processes which can not be reversed when the fruits are restored to normal temperature. Such fruits often will decay before ripening. Bananas are particularly sensitive, failing to ripen properly if kept for a few days at temperatures below 56° F. Similar effects have been observed on flower and onion bulbs and on pears and potatoes. Knowledge of proper storage temperatures and the effects of specific temperature ranges not only prevents spoilage of fruits and vegetables, but has permitted the production of desirable physiological changes such as inversion of sugar in fruits and the control of flowering or bloom in plants to increase the yield of seed or for commercial forcing of the bloom. Some recommended storage temperatures follow: Bananas, ripening, 62° to 70° F., and holding ripe bananas, 56° to 60° F.; lemons, 55° to 58° F.; oranges, 34° to 38° F.; mature green pineapples, 50° to 60° F., ripe, 40° to 45° F.; mature green tomatoes, 55° to 70° F., ripe, 40° to 50° F. *The Scientific Monthly, 62,* 173 (1946).

EYES AND MALNUTRITION—A recent study of eye disorders in Marine and Naval personnel in the Southwest Pacific substantiates experimental animal and clinical studies on the incidence of ocular disturbances as early signs of nutritional deficiency. The majority of the men examined had been in combat for six to eighteen months, subsisting chiefly on canned foods of limited variety and subject to unavoidable vitamin and mineral deficiencies. The major complaint was loss of visual acuity caused by an aggravation of a pre-existing trivial degree of near-sightedness. Administration of vitamin D and calcium appeared to result in a reduction or arrest of the progressive myopia in more than 50 per cent of the patients. Similar therapy was found effective in clearing up a condition of "inflamed eyes," or allergic conjunctivitis, a disorder characterized by itching, sensitivity to light and a catarrhal discharge. Night blindness, an accepted sign of nutritional deficiency, of course responded to vitamin therapy. Other ocular disturbances, traced to infectious or toxic causes, responded well to combined medical and nutrition therapy. Common to all the patients was the fact that apart from the eye signs there was no clinical evidence of malnutrition in any of the other tissues. All of the men appeared to be healthy, the systemic deficiency apparently being latent. Nutritious diets, fortified by supplements of vitamins A, B, C and particularly D, were highly effective in clearing up the eye disturbances. Particular attention was paid to the milk intake, one quart daily being advised in order to provide the optimum amount of calcium. The studies lead to the conclusion that examination of the eyes may serve as a guide in the detection of latent nutritional deficiency diseases. *Bull. N. Y. Acad. Med. 22,* 217 (1946).

RIBOFLAVIN METABOLISM — Interesting data have been obtained in studies on the riboflavin requirement of rats as influenced by diet. It was found that rats on a low protein diet showed a relatively large excretion of riboflavin in the urine and were unable to store riboflavin in their organs. Rats on high protein, high fat diets needed at least twice as much riboflavin as rats on a "normal" diet for the maintenance in organs and urine of an equal level of riboflavin. Rats on low fat diets needed only about half as much riboflavin. The different dietetic riboflavin requirements of rats kept on different diets have been demonstrated to be due to differences in the amounts of riboflavin which are synthesized in a form available to the organism in the intestine. *J. Biol. Chem., 162, 267* (1946).

NUTRITION AND AGING—Results of a ten-year study at a clinic in the South have revealed important facts concerning the incidence and effects of continued malnutrition. It has been shown that various conditions judged to be simply a part of growing old actually are brought on by lack of proper nutrition. This is particularly true in the area studied where many people in the lower income brackets work for long hours in fields, factories or mines. Women, frequently during childbearing, spend long hours caring for homes and children as well as working outside the home. Nutritional deficiency has been found to be most common in this group. In the cases observed, most of the women suffered from pellagra, nervousness and hallucinations, sore mouth and tongue, severe pain in feet and legs and loss of weight. One woman of 30 was judged by her appearance to be 50. Another who was 70, psychotic and physically ill, began to lead a vigorous and normal life following nutritional therapy and the maintenance of proper dietary habits. Although the exact role played by nutritive failure in the aging process is still obscure, it is pointed out that proper nutrition may prevent signs of premature aging and may frequently restore aging persons to an active and productive existence. *J. Gerontology 1,* 33 (1946).